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Vascular Permeability Changes in the Central Nervous System of Rats with Hyperacute Experimental Allergic Encephalomyelitis Induced with the Aid of a Substance from *Bordetella pertussis*

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Development of hyperacute experimental allergic encephalomyelitis in Lewis rats after intraperitoneal administration of a mixture of guinea pig spinal cord emulsion and pertussigen from *Bordetella pertussis* was accompanied by an increase in vascular permeability in the central nervous system. The increased permeability was most striking in the spinal cord and seemed to be associated with the ascending development of paralysis. Rats that had completely recovered from paralysis did not have any increased permeability in the central nervous system. Rats which developed paralysis after inoculation with either guinea pig spinal cord emulsion alone or with complete Freund adjuvant had only a small degree, if any, of increased permeability in the vascular system of the central nervous system.

Lee and Olitsky were first to report that pertussis vaccine administered intraperitoneally (i.p.) into mice would enhance development of encephalomyelitis after intracutaneous administration of mouse brain antigens in complete Freund adjuvant (CF; 7). Levine et al. (15) showed that *Bordetella pertussis* cells and fractions rich in histamine-sensitizing factor (HSF) increased the encephalitogenic activity of guinea pig spinal cord emulsions when administered to Lewis rats. Furthermore, *B. pertussis* cells or fractions of these cells accelerated the onset of experimental allergic encephalomyelitis (EAE) and changed the histological features in the central nervous system (CNS) lesion, thus producing a hyperacute type of EAE (14, 15). When EAE was induced by giving spinal cord emulsions in complete Freund adjuvant, the cellular infiltrate in the CNS was mainly of the mononuclear cell type, but when *B. pertussis* vaccine was used, a polymorphonuclear infiltrate and fibrin deposition were also observed (14). Levine et al. (15) felt that this action of *B. pertussis* cells was due to HSF or closely associated with it.

HSF increases histamine sensitivity in mice and rats, induces lymphocytosis, protects mice from intracerebral challenge with virulent *B. pertussis*, increases antibody response to many antigens, and accelerates some autoimmune diseases (18). The substance consists mainly of protein and, possibly, lipid (8, 17, 25). It is heat labile (inactivated by 80°C for 1/2 h) and is destroyed by certain protein-denaturing agents and some proteolytic enzymes (18). Because this substance has been referred to in the literature by different names, we have proposed the unifying name of "pertussigen" (J. J. Munoz, Fed. Proc. **35**:813, 1976), and it will thus be referred to in this paper.

The mechanism by which pertussigen induces hyperacute EAE is not known, but it has been postulated that the vascular permeability in the CNS may be involved (4, 19). In this paper we explore this possibility further.

MATERIALS AND METHODS

Preparation of pertussigen. Pertussigen was extracted and obtained in two degrees of purity from *B. pertussis* cells by the methods of Munoz et al. (20). The majority of the work was done with a 1 M NaCl-0.05 M sodium pyrophosphate extract of acetone-dried *B. pertussis* cells (BPE; 20), and some experiments were performed with a highly purified material obtained by electrophoresis of soluble material on a sucrose density-gradient column (pool B; 20).

Animals. Female Lewis rats weighing 100 to 125 g were purchased from Microbiological Associates, Walkersville, Md. Spinal cords and spleens were obtained from adult Hartley strain guinea pigs raised in our laboratory.

Preparation of encephalitogenic antigen. Spinal cords and spleens from guinea pigs and brains from rats were processed by the method of Levine et al. (15) for spinal cords. Briefly, a suspension of tissue was made in physiological saline (100 mg/ml), heated

at 60°C for 45 min, and emulsified by passing it repeatedly through a 19-gauge needle. A preparation of purified basic protein from rat brains was kindly supplied by M. Kies, National Institutes of Health, Bethesda, Md.

Induction of hyperacute EAE. Hyperacute EAE was induced by the i.p. route as described by Levine et al. (14, 15). Rats received i.p. 200 mg of antigen mixed with an appropriate amount of *B. pertussis* fraction in phosphate-buffered saline (PBS). *B. pertussis* fractions were tested at three dose levels. The animals, kept in plastic boxes in groups of 3 to 5, were observed daily for signs of EAE.

Method of scoring EAE. Severity of paralysis was rated from 0 to 4 as follows: 0 = no symptoms; 1 = rough hair coat, slight ataxia, and slight paresis of tail and hind legs; 2 = definite paresis of tail and hind legs, but still able to move about on all four legs; 3 = complete paralysis of tail and both hind legs, but capable of moving around by means of front legs; 4 = totally paralyzed, lying on side.

Histology. Brain and spinal cords were removed, fixed in 10% neutral buffered Formalin, sectioned, and stained with either hematoxylin-eosin (H-E) stain or phosphotungstic acid hematoxylin (PTAH) stain. Sections were examined for histopathology by light microscopy.

Test for vascular permeability. Vascular permeability in the spinal cord, brain, and other tissues was measured by the double isotope method of Leibowitz and Kennedy (10) as described in a previous publication (3).

Each rat received intravenously 5 μCi of ^{131}I-labeled human serum albumin in 1 ml of saline 24 h before receiving a second intravenous injection of 5 μCi of ^{125}I-labeled human serum albumin in 1 ml of saline. Exactly 5 min later, 20 μl of blood was taken from the infraorbital sinus, the rats were sacrificed by decapitation, and the spinal cords and brains were removed. Starting at the cervical end, the cords were cut into four segments of approximately equal length (2.5 cm). Brain (either whole brain or a lateral half obtained by section through a sagittal plane) and spinal cord segments were weighed, and then the quantity of ^{131}I and ^{125}I in each tissue sample and blood sample was determined in a dual channel γ-scintillation spectrometer (Nuclear-Chicago Corp.). Briefly, the technique is performed as follows. Tissue and blood specimens are counted for both ^{125}I and ^{131}I activity. ^{125}I activity is corrected for counts due to ^{131}I "breakthrough" into the ^{125}I channel. For each tissue sample the content of ^{131}I and ^{125}I is expressed in arbitrary units, "blood equivalents" (BE) for both isotopes: BE = (counts per minute/gram of tissue)/(counts per minute/milliliter of blood) × 100. Under the experimental conditions, ^{125}I is essentially intravascular and the ^{125}I-BE value represents a measurement of tissue blood volume. ^{131}I is both intravascular and extravascular, and thus the difference between the ^{125}I-BE value and the ^{131}I-BE value for a particular tissue specimen is an indication of how much fluid has leaked out of the vascular bed and is expressed as extravascular blood equivalents (EVBE), i.e., ^{131}I-BE − ^{125}I-BE = EVBE. This technique allows permeability changes to be measured independently of alterations in tissue blood volume.

In one experiment 10 mg of Evans blue dye in 1 ml of saline was administered intravenously 4 h before sacrificing the rats to visualize increases in vascular permeability in the spinal cord and brain.

RESULTS

Preliminary work. An experiment was performed to determine whether under our laboratory conditions Lewis rats would develop hyperacute EAE when given guinea pig spinal cord mixed with crude BPE. Eight rats which received an emulsion of 200 μg of BPE and 200 mg of cord were all severely paralyzed 7 to 8 days after inoculation. In a group of eight rats which received only 200 mg of cord, three rats developed 1+ or 2+ paralysis at 10 to 13 days, while another group which received 200 μg of BPE and 12 to 20 mg of cord did not develop any paralysis.

Vascular permeability changes in rats with hyperacute EAE. The marked increase in vascular permeability in the spinal cord of rats 7 days after receiving guinea pig spinal cord homogenate and pertussigen is shown in Table 1. Administration of 200 μg of BPE, along with 200 mg of guinea pig spinal cord homogenate, produced a 34-fold increase in the EVBE value for the spinal cord, a 6.5-fold increase for the brain, and a 3.4-fold or less for other tissues.

In Table 2 the vascular permeabilities of the spinal cord and brain of rats receiving a variety of sensitizing treatments are compared. These data show that control groups which received PBS, PBS + BPE, guinea pig spleen + BPE, or guinea pig cord did not differ to any significant degree in EVBE values over a 14-day period.

TABLE 1. *Comparison of changes in tissue permeability induced by BPE in rats inoculated with a homogenate of guinea pig (GP) spinal cord*

Treatment	Tissue permeability expressed as EVBE (mean ± SEM)[a]					
	Brain	Spinal cord	Thigh muscle	Kidney	Lung	Liver
200 mg of GP cord[b]	0.22 ± 0.07	0.68 ± 0.16	3.16 ± 0.26	4.55 ± 0.75	15.58 ± 1.33	2.89 ± 0.65
200 mg of GP cord + 200 μg of BPE[c]	1.44 ± 0.21	23.26 ± 4.29	5.81 ± 0.70	15.43 ± 2.14	19.14 ± 3.04	5.35 ± 0.70

[a] SEM, standard error of the mean.
[b] Four rats in treatment group.
[c] Five rats in treatment group.

TABLE 2. *EVBE values for the brain and spinal cord of rats inoculated with various tissue emulsions and crude pertussigen (BPE) to produce hyperacute EAE*[a]

Tissue tested	Sensitizing treatment	EVBE at following day after sensitizing treatment:						
		1	5	6	7	8	12	14
Brain	GP cord	0.75 ± 0.07[b] (9)[c]	0.33 ± 0.11 (8)	0.95 ± 0.06 (4)	0.55 ± 0.13 (4)	0.83 ± 0.15 (9)	0.97 ± 0.16 (9)	0.56 ± 0.16 (4)
	GP cord + BPE	0.74 ± 0.09 (9)	0.72 ± 0.15 (8)	1.55 ± 0.22 (4)	2.15 ± 0.40 (4)	4.14 ± 0.73 (12)	2.68 ± 0.16 (4)	0.56[d] (1)
	GP spleen + BPE		0.50 ± 0.10 (4)		0.64 ± 0.07 (4)			0.32 ± 0.18 (4)
	Rat brain + BPE		0.48 ± 0.11 (4)		4.38 ± 0.37 (4)			1.60 ± 1.20[e] (2)
	PBS + BPE					1.26 ± 0.26 (5)		
	PBS	0.78 ± 0.08 (5)					0.74 ± 0.08 (5)	
Cord, 1st section (cervical end)	GP cord	1.47 ± 0.14	1.07 ± 0.27	2.57 ± 0.44	1.14 ± 0.23	3.65 ± 0.82	4.33 ± 0.85	0.89 ± 0.24
	GP cord + BPE	1.65 ± 0.16	1.57 ± 0.42	14.21 ± 8.28	27.56 ± 3.61	47.31 ± 3.04	36.17 ± 7.84	0.40
	GP spleen + BPE		1.33 ± 0.73		1.26 ± 0.30			0.61 ± 0.23
	Rat brain + BPE		0.94 ± 0.33		34.21 ± 1.42			1.04 ± 0.38
	PBS + BPE					3.04 ± 0.72		
	PBS	1.28 ± 0.08					2.49 ± 0.44	
Cord, 4th section (caudal end)	GP cord	5.93 ± 0.62	5.23 ± 1.26	7.73 ± 0.58	6.64 ± 1.46	13.78 ± 3.47	8.09 ± 1.07	3.98 ± 0.71
	GP cord + BPE	6.79 ± 0.70	11.93 ± 1.47	25.76 ± 7.27	26.07 ± 2.07	27.18 ± 3.26	22.55 ± 4.62	2.54
	GP spleen + BPE		8.38 ± 1.36		5.47 ± 0.50			5.54 ± 0.62
	Rat brain + BPE		9.08 ± 1.21		26.75 ± 1.56			5.04 ± 1.15
	PBS + BPE					7.07 ± 0.80		
	PBS	4.01 ± 0.59					7.61 ± 1.18	

[a] For simplicity, only data for the brain and first and fourth sections of the spinal cord are shown. The permeability in the second and third sections was quite similar to that in the first section except on day 6 when it was more like that in the fourth section. GP, Guinea pig.

[b] Values are expressed as mean EVBE ± standard error of the mean.

[c] Numbers in parentheses represent number of rats in treatment group.

[d] This rat had a 1+ paresis on day 14, but had been 3+ on day 9.

[e] One rat had a 1+ paresis on day 14, but had been 3+ on day 10; the other had never shown any paresis.

Among these groups, paresis in the tail and hind limbs developed only in 4/13 of the rats which had received guinea pig cord homogenate. This paresis began on about day 9 or 10 and lasted only a few days. On the other hand, vascular permeability was markedly increased in the spinal cord of rats receiving spinal cord and BPE. Increased permeability in this group started on day 5 in the fourth segment of the spinal cord, and by day 6, the vascular permeability was high throughout the spinal cord. The permeability of the vessels remained high through day 12. In the same group, one of four rats used on day 6 had a 3+ paralysis, and 19/20 of the rats used on the days 7, 8, and 12 had 3+ or 4+ paralysis. It was of interest that at day 5 the greatest permeability was observed in the fourth segment of the cord. On days 8 and 12, however, the permeability was greater in the upper sections. In one experiment in which the permeability of the cord was visualized in paralyzed rats by an intravenous injection of Evans blue, it was also apparent that the fourth segment of the cord was more permeable to the dye than were the upper sections, at least in the early stages of paralysis, which suggests a relationship of increased permeability with the ascending type of paralysis observed in EAE.

Administration of 200 mg of rat brain homogenate, along with 200 μg of BPE, also produced hyperacute EAE. By day 5, the fourth segment of the spinal cord showed only a very slight increase in vascular permeability, but by day 7 a striking increase in permeability throughout the CNS was observed. In this group, all four rats had a paralysis score of 3+ on day 7. Rats that received rat brain emulsions + BPE, in general, developed a severe hyperacute EAE and most died within 9 to 10 days.

Disappearance of vascular permeability and recovery from hyperacute EAE. In many experiments it was found that rats sensitized with guinea pig spinal cord or rat brain plus BPE that did not develop more than 3+ paralysis and a few of those which developed even 4+ paralysis recovered from the disease. There were only two such animals surviving at day 14 (Table 2), and elevated vascular permeability levels were not demonstrable in either of them. To further elucidate the relationship between EAE and vascular permeability, we decided to compare rats undergoing hyperacute EAE, induced by BPE, and classical EAE, induced by giving guinea pig spinal cord in an emulsion with CF, and to measure the permeability before, during, and after development of EAE. Twenty-five rats received i.p. 200 mg of cord + 200 μg of BPE, and 25 rats received 10 mg of guinea pig spinal cord intradermally in an emulsion with CF. Fifteen controls received i.p. 200 mg of cord in saline. The rats were checked daily for development of EAE, and the vascular permeability in spinal cords and brains was measured by the double isotope method at 1, 8, 12, 15, and 21 days after sensitization. Many of the rats which received cord and BPE died of severe EAE after day 8 (13 dead out of 15 rats). The two rats that survived had 3+ and 4+ paralysis on day 11. Because there were only two survivors in this group of rats, permeability tests were not run for this group on days 12 and 15. The inoculation with cord in CF produced a milder, less acute form of EAE. Symptoms appeared on about day 15 and had disappeared by day 21. The results of the permeability measurements are summarized in Table 3. In parallel with the severity of the paralysis which developed, vascular permeability in the brain and spinal cord was markedly increased on day 8 in the group of animals receiving cord and BPE but not in those which received the antigen in CF. Of the 20 rats kept 8 days or longer and that had received cord and BPE, all developed 3+ or 4+ paralysis. From this same group, of the 15 rats kept longer than 8 days, 13 died and only 2 recovered. In 10 rats which received the cord in CF and were kept 15 days or longer, 7 of 10 developed 1+ or 2+ paralysis and 1 had a score of 3+. Paralysis had disappeared by day 21. The vascular permeability in the brain and spinal cord of these animals was only slightly increased on days 15 and 21 (Table 3).

There was a direct relationship between the dose of pertussigen given to the rats with the encephalitogenic antigen and the severity of the hyperacute EAE which subsequently followed. As mentioned above, of 15 rats that had received 200 μg of BPE and 200 mg of guinea pig spinal cord emulsion, only 2 survived to 21 days. If the amount of pertussigen was reduced, the number of animals surviving improved considerably, even though the dose of encephalitogenic antigen remained the same. The course of paralysis in two groups of rats is illustrated in Fig. 1 and 2. In Fig. 1 the rats received 200 mg of cord + 20 μg of purified pertussigen (pool B), and in Fig. 2 the rats received only 2 μg of purified pertussigen + 200 mg of cord. Of those rats receiving 20 μg of pertussigen, one died after becoming totally paralyzed, two developed 4+ paralysis and then improved and eventually recovered, and one never developed symptoms of EAE. The paralysis scores of the four rats that received the cord mixed with only 2 μg of pertussigen were 3+, 3+, 2+, and 1+. All of these rats had completely recovered by day 20.

Histopathology studies. The results of histological studies on spinal cords of rats that had

TABLE 3. *Comparison of EVBE values in CNS of rats inoculated with guinea pig spinal cord emulsion and crude pertussigen (BPE) or CF*

Tissue tested	Sensitizing treatment[a]	EVBE at following day after sensitizing treatment:				
		1	8	12	15	21
Brain	Cord + BPE	0.59 ± 0.11[b]	3.59 ± 0.68	—	—	0.85 ± 0.23 (2)
	Cord	0.73 ± 0.14	—	0.82 ± 0.19	—	0.61 ± 0.07
	Cord + CF	0.58 ± 0.06	0.85 ± 0.09	0.62 ± 0.12	1.02 ± 0.15	1.67 ± 0.27
Cord, 1st section (cervical end)	Cord + BPE	1.38 ± 0.22	53.01 ± 4.23	—	—	3.38 ± 2.16
	Cord	1.44 ± 0.23	—	3.59 ± 1.06	—	1.56 ± 0.28
	Cord + CF	1.36 ± 0.23	1.00 ± 0.12	1.76 ± 0.73	5.62 ± 0.81	6.14 ± 1.01
Cord, 2nd section	Cord + BPE	3.32 ± 0.72	54.05 ± 5.94	—	—	3.56 ± 1.59
	Cord	3.43 ± 0.61	—	4.62 ± 1.29	—	2.49 ± 0.41
	Cord + CF	4.68 ± 1.56	3.16 ± 0.75	3.24 ± 0.59	6.08 ± 1.70	7.40 ± 0.65
Cord, 3rd section	Cord + BPE	1.77 ± 0.37	56.62 ± 7.48	—	—	2.47 ± 0.63
	Cord	2.32 ± 0.44	—	3.30 ± 1.04	—	1.82 ± 0.19
	Cord + CF	2.24 ± 0.30	1.95 ± 0.35	2.72 ± 1.15	9.22 ± 4.38	5.50 ± 0.85
Cord, 4th section (caudal end)	Cord + BPE	7.07 ± 1.27	33.46 ± 6.28	—	—	7.37 ± 2.26
	Cord	5.89 ± 0.73	—	7.28 ± 1.00	—	4.93 ± 0.69
	Cord + CF	8.12 ± 0.57	5.97 ± 0.91 (4)	6.57 ± 0.91	10.37 ± 3.46	7.82 ± 1.51

[a] Sensitizing treatment: cord + BPE = 200 mg (wet weight) of guinea pig spinal cord emulsion + 200 μg of BPE in 4 ml of PBS, i.p.; cord = 200 mg of guinea pig spinal cord in 4 ml of PBS, i.p.; cord + CF = 10 mg of guinea pig spinal cord in 0.025 ml of PBS + 0.025 ml of CF emulsified together and given intradermally.

[b] EVBE was determined as previously described (3), and the values given are the mean ± standard error of the mean. Five rats per group were used except where noted by numbers in parentheses.

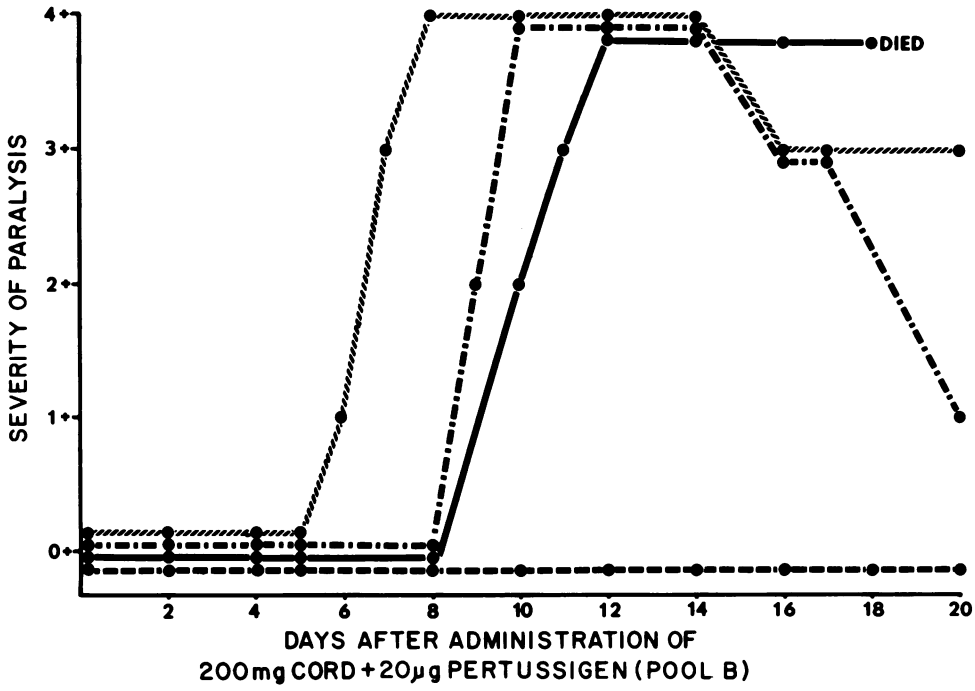

FIG. 1. *Onset and recovery from hyperacute EAE after sensitization of rats with guinea pig spinal cord emulsion and a high dose of pertussigen (20 μg of pool B).*

received 200 μg of BPE mixed with either 200 mg of rat brain, 200 mg of guinea pig spinal cord, 200 mg of guinea pig spleen, or 100 μg of rat purified basic protein are given in Table 4.

Rats receiving guinea pig cord + BPE or rat brain + BPE developed by day 8 a marked

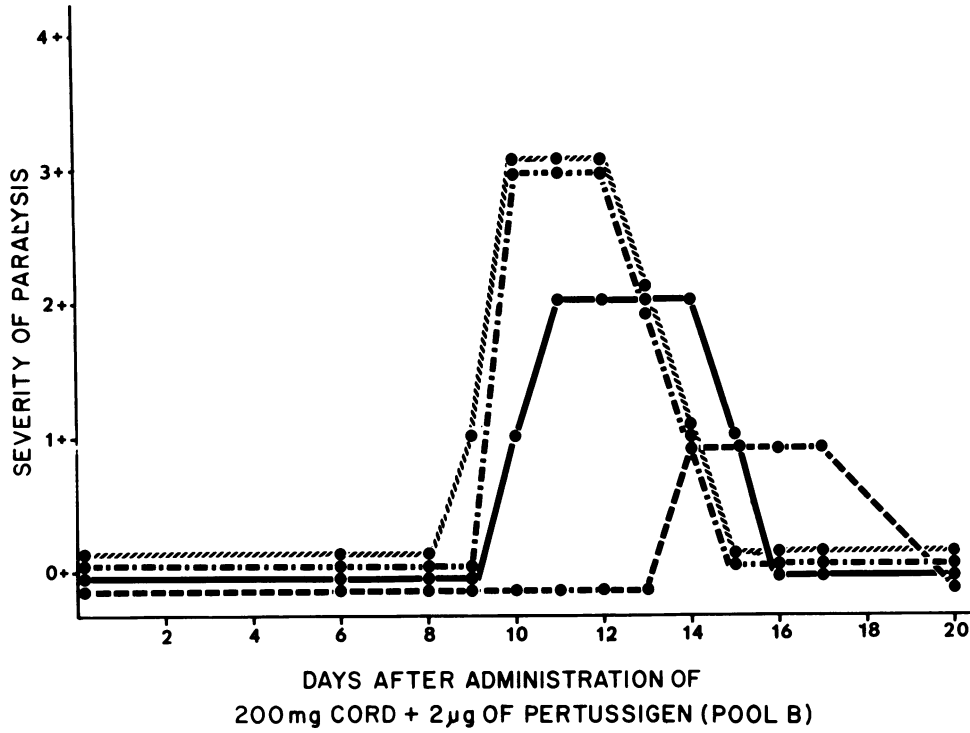

FIG. 2. *Onset and recovery from hyperacute EAE after sensitization of rats with guinea pig spinal cord emulsion and a low dose of pertussigen (2 μg of pool B).*

TABLE 4. *Histological changes in the spinal cord of rats receiving different mixtures*

Sensitizing treatment	Rat no.	5[a]				8[a]				15[a]			
		M[b]	P	F	N	M	P	F	N	M	P	F	N
GP[c] cord only	1	–	–	–	–	4+	2+	–	–	2+	1+	–	–
	2	–	–	–	–	3+	1+	–	–	3+	1+	–	–
	3	–	–	–	–	4+	2+	–	–	1+	–	–	–
GP cord + BPE	1	–	–	–	–	3+	2+	1+	1+	D[d]	D	D	D
	2	–	–	–	–	3+	3+	1+	1+	D	D	D	D
	3	±	±	–	–	D	D	D	D	D	D	D	D
GP spleen + BPE	1	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	1+	1+	–	–
Rat basic protein + BPE	1	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	±	–	–	–	–	–	–	–
	3	–	–	–	–	±	–	–	–	–	–	–	–
Rat brain + BPE	1	2+	1+	–	–	2+	3+	1+	1+	D	D	D	D
	2	1+	1+	–	–	3+	4+	1+	1+	D	D	D	D
	3	–	–	–	–	D	D	D	D	D	D	D	D

[a] Days after sensitization.

[b] M, Mononuclear cells; P, polymorphonuclear; F, fibrin; N, neuronal destruction. Nine rats were employed per treatment group, and three were sacrificed at each of days given above. The readings are subjective averages of the predominance of the elements in three separate sections of the cord.

[c] GP, Guinea pig.

[d] D, Rat died.

diffused mononuclear cell infiltration with scattered polymorphonuclear cells in the grey and white matter. Acute vasculitis with some polymorphonuclear cell infiltration of vessel walls and some focal hemorrhages were seen. Some neurons were in varying stages of degeneration,

and few neurons were infiltrated with polymorphonuclear leukocytes (Fig. 3). Perivascular fibrin deposition (revealed by PTAH stain) was also seen on day 8. At 5 days, the rats that received guinea pig cord + BPE showed no histological changes in the cord or brain; however, the animals that received rat brain + BPE already showed slight mononuclear and polymorphonuclear leukocyte infiltration at this time.

Rats that received guinea pig cord alone also showed histological changes in the cord that were pronounced by day 8. The pathology in this group consisted mainly of a peripheral (meningeal) and focal perivascular mononuclear infiltration. An occasional polymorphonuclear leukocyte was seen, but the cell infiltrates consisted mainly of lymphocytes and histiocytes within the meninges and about the major nerve trunks of the cauda equina.

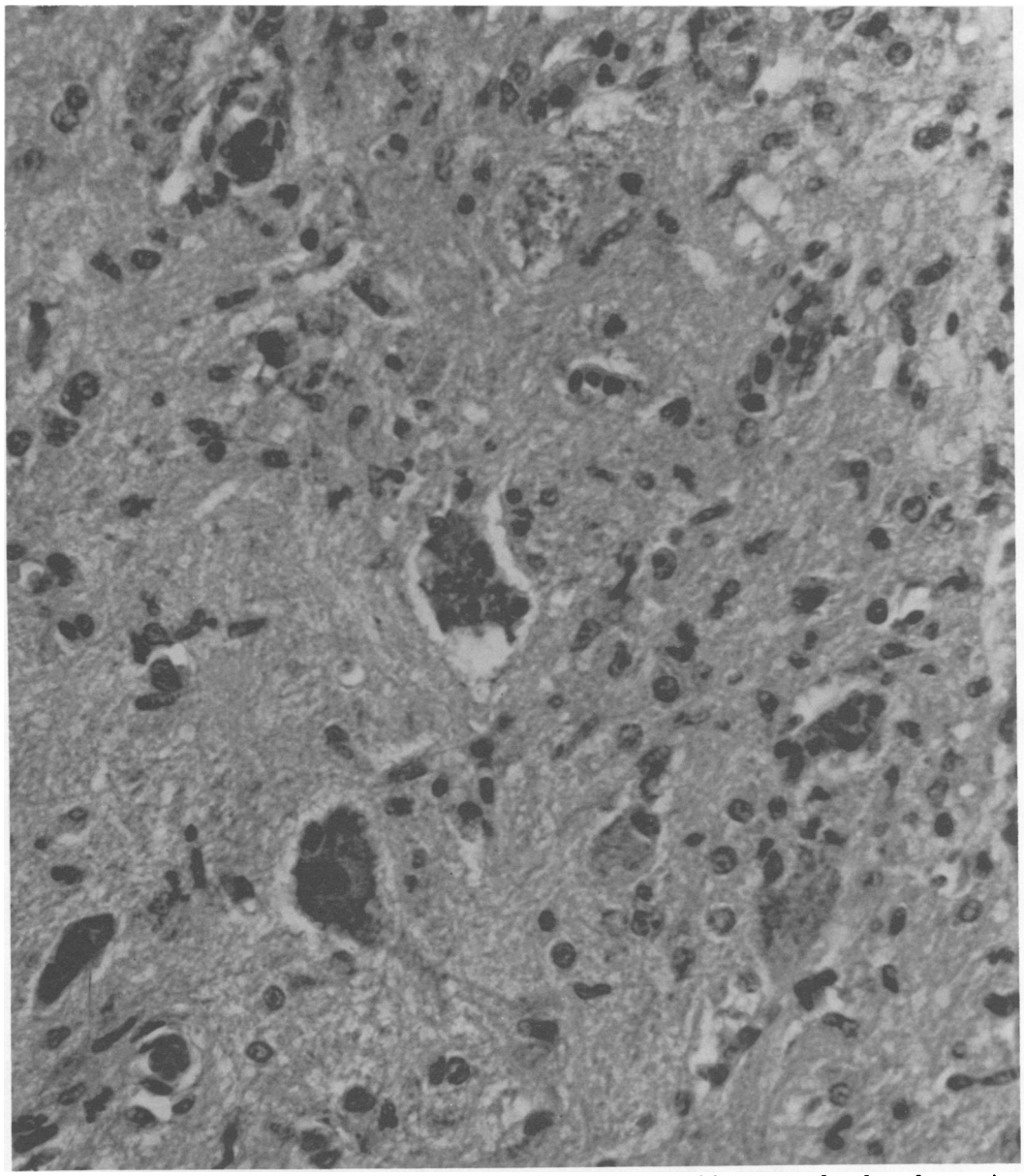

FIG. 3. *Photomicrograph (× 500) of a longitudinal section of spinal cord from a paralyzed rat that received rat brain homogenate + BPE 8 days before removing the spinal cord. Note the neuron infiltrated with neutrophils.*

Animals receiving guinea pig spleen + BPE or rat basic protein + BPE had no significant histological changes in the spinal cord. One animal which had received guinea pig spleen + BPE showed on day 15 some unexplained minimal perivascular cellular infiltration.

The contrast between the pathology seen in animals treated with guinea pig cord + BPE and guinea pig cord alone is illustrated in Fig. 4. These photomicrographs were taken of longitudinal sections from the mid part of the cord.

Brains of animals which received guinea pig spinal cord alone had a slight mononuclear infiltrate on days 8 and 15 after sensitization. Those rats that received guinea pig spinal cord + BPE or rat brain + BPE also showed mild mononuclear infiltrates with rare polymorphonuclear cells.

DISCUSSION

The work of Levine et al. (14, 15) established that *B. pertussis* vaccine and semipurified fractions of the so-called HSF from this bacterium induced in Lewis rats an accelerated form of EAE that was characterized by early onset of paralysis and a characteristic infiltrate which contained many polymorphonuclear leukocytes and fibrin. We have proposed that the active material in *B. pertussis* which produces histamine sensitization, lymphocytosis, hypoglycemia, and hyperacute EAE be called "pertussigen" (J. J. Munoz, Fed. Proc. **35**:813, 1976).

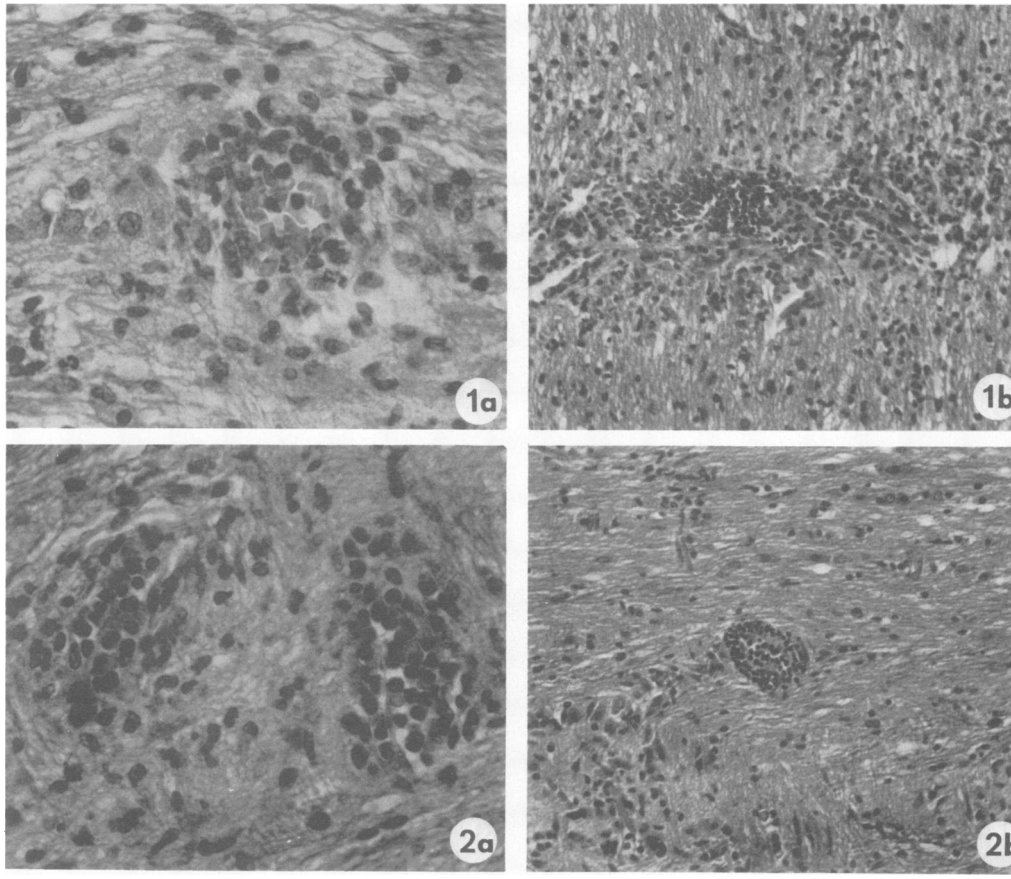

FIG. 4. *Photomicrographs of longitudinal sections from spinal cords of rats that had received: (1) 200 mg of guinea pig spinal cord homogenate + 200 μg of BPE which produced a diffuse cellular infiltrate and many neutrophils about the vessels. Note neutrophils within the infiltrate in 1a (× 500) and inflammatory cells disrupting vessel wall in 1b (× 175); (2) 200 mg of guinea pig spinal cord homogenate alone which resulted in a predominantly meningeal and perivascular infiltrate without a diffuse inflammatory component. Note the inflammatory infiltrate is composed exclusively of mononuclear cells, predominantly lymphocytes (1a, × 500), and is restricted to the Virchow-Robbin space in 2b (× 175). The spinal cords were obtained 8 days after sensitization, and sections were stained with H-E stain.*

The manner by which pertussigen induces accelerated EAE is not well understood. Two actions of this substance may be involved: (i) the adjuvant action, and (ii) enhancement of the ability of vasoactive agents to increase vascular permeability. Levine et al. (11, 12, 14) and Paterson (22) have shown that edema and fibrin deposition occur in the spinal cord of rats with EAE. In the present work, we have shown conclusively that pertussigen increased vascular permeability in the spinal cord during the onset of paralysis in hyperacute EAE. Furthermore, this permeability appeared first in the lower region of the cord and then throughout the cord. In severe cases of EAE, vascular permeability was also increased in the brain. In our laboratory, 10 mg of guinea pig spinal cord emulsion mixed with CF adjuvant induced mild EAE in Lewis rats. Paralysis started at about day 15 in contrast to days 6 to 9 in rats receiving pertussigen and 200 mg of cord. Furthermore, the increase in vascular permeability in brains and cords of rats receiving spinal cord in CF was only slight on days 15 and 21. A few Lewis rats receiving 200 mg of guinea pig cord alone also developed mild paralytic EAE by day 12 (1+ or 2+ response). The brains and spinal cords of these animals did not show much increase in vascular permeability, although of seven rats which had developed some paralysis, three had an elevated permeability in the posterior (fourth) segment of the spinal cord. It is important to emphasize that almost invariably rats that develop up to a 3+ paralysis recovered fully within 1 to 2 weeks after onset of paralysis. The mechanism of this recovery is intriguing and should be thoroughly investigated. We know that rats which had received pertussigen and developed 3+ or 4+ paralysis no longer showed increased vascular permeability of the spinal cord (Tables 2 and 3) when they recovered from paralysis. Perhaps the recovery from EAE results from the production of suppressor cells that protect nervous tissue from the effects of either specifically sensitized cells or antibody. Adoptive transfer of viable lymph node cells from donor Lewis rats, made tolerant to clinical EAE, rendered syngenic recipients unresponsive to a challenge with basic protein and CF adjuvant (29). Swanborg has postulated that a natural, life-long stimulation of suppressor cells may be one mechanism for preventing autoimmune diseases (26). Other types of EAE suppression do not involve regulatory cells but apparently are mediated through antibodies induced by encephalitogens (1, 23).

The histological studies showed, as previously described (14, 15, 27), that histopathological changes without clinical signs can occur in animals receiving encephalitogenic material alone. The lesions in this case were mostly peripheral, and the cellular infiltration was mainly of the mononuclear type (lymphocytes and histiocytes). In these animals no significant increase in vascular permeability of the spinal cord was observed. In contrast to this, animals that received encephalitogenic material (guinea pig spinal cord or rat brain) mixed with pertussigen developed a more central cellular infiltration, both in the grey and white matter, consisting of mononuclear cells and polymorphonuclear leukocytes. Acute vasculitis with extravascular fibrin deposition and some hemorrhages were also detected. In severe cases, neuronophagia was also observed. We have not studied the histological changes in rats that recovered from mild forms of EAE, but since complete recovery of motor activity occurred, neuronal destruction is probably minimal.

We are inclined to think that the initial paralysis in hyperacute EAE is the result of edema after a marked increase in vascular permeability of spinal cord capillaries and also due to an acute vasculitis. In our work the onset of paralysis corresponded to the onset of increased permeability in the cord, and the ascending type of paralysis seemed to parallel the increase in permeability which first appeared in the caudal end of the cord and then increased in the mid and cervical segments.

Histological changes induced in Lewis rats receiving guinea pig spinal cord without adjuvants were, in most cases, not sufficient to produce paralysis; these mostly peripheral and focal changes did not seem to explain paralysis. In rats receiving pertussigen + cord, the histological changes were more diffusely distributed throughout the grey and white matter. It seems to us that the increase in permeability of capillaries may be responsible for this cellular distribution.

Paterson (22) has postulated a pathway of inflammation in EAE which is initiated by the release of "lymphokines," including permeability factors, after interaction of sensitized lymphocytes with target antigens. This induces a cascade of events involving accumulation of fibrinogen and other clotting factors in the target area, fibrin formation, then initiation of fibrinolytic activity, and release of biologically active polypeptides, with the end result being edema. The ability of pertussigen to enhance vascular permeability may act synergistically with these soluble mediators proposed by Paterson to augment the distribution of fibrinogen and clotting factors into the lesions and enhance the devel-

opment of edema.

The relationship of fibrin formation to the development of hyperacute EAE is a moot point. Levine and Wenk (14) reported a striking accumulation of perivascular fibrin in the CNS lesions of hyperacute EAE. However, Levine et al. (15) have observed early onsets of paralysis in the absence of fibrin. Levine has suggested that the term hyperacute should be applied to EAE when fibrin is present and all or most of the rats die (personal communication). Using a highly sensitive immunofluorescence method for detecting fibrin, Paterson (22) has detected fibrin deposition in both hyperacute and classical EAE of rats and in animals that did not have clinical disease.

Since pertussigen has an adjuvant effect on cellular hypersensitivity (24) and stimulates the production of antibodies in different immunoglobulin classes, such as immunoglobulin G, immunoglobulin M, and immunoglobulin E (5, 9, 28), it likely acts through an adjuvant action to increase the encephalitogenic effects of guinea pig spinal cord antigen. It must be pointed out that, to date, serum antibodies have not been found to be involved in EAE (13, 21).

An important role of pertussigen could be the blockade of an adrenergic receptor (6) which results in an altered reactivity of the vessels to vasoactive substances. In this respect it should be noted that susceptibility of rats to EAE can also be increased by removal of adrenal glands (16).

The adrenergic blockade which pertussigen induces (6, 18) may, through its effects on metabolism and the neuro-endocrine system, diminish the rats' ability to counteract or compensate for the vascular effects produced by endogenously released vasoactive amines such as histamine and/or serotonin. These amines are released directly (from mast cells and basophils) or indirectly (from platelets following release of platelet-aggregating factor from basophils [2]) when sensitized cells are challenged with antigen. If such a release occurs in the proximity of capillaries of the spinal cord and brain of animals in which an adrenergic blockade exists, antibody and/or sensitized cells may penetrate the parenchyma of the spinal cord and brain more readily and in larger amounts and thus accentuate the disease. Thus, the mechanism by which pertussigen enhances EAE in rats may be twofold: (i) an increased antibody or cellular response to spinal cord antigen, and (ii) a blockade of adrenergic receptors which makes the animals more susceptible to endogenously released vasoactive substances, thus increasing vascular permeability in the spinal cord and brain where these substances would be released as a result of antigen-antibody or antigen-sensitized cell reactions.

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